

A humanized gnotobiotic mouse model of host–archaeal–bacterial mutualism

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Our colons harbor trillions of microbes including a prominent archaeon, *Methanobrevibacter smithii*. To examine the contributions of Archaea to digestive health, we colonized germ-free mice with *Bacteroides thetaiotaomicron*, an adaptive bacterial forager of the polysaccharides that we consume, with or without *M. smithii* or the sulfate-reducing bacterium *Desulfovibrio piger*. Whole-genome transcriptional profiling of *B. thetaiotaomicron*, combined with mass spectrometry, revealed that, unlike *D. piger*, *M. smithii* directs *B. thetaiotaomicron* to focus on fermentation of dietary fructans to acetate, whereas *B. thetaiotaomicron*-derived formate is used by *M. smithii* for methanogenesis. *B. thetaiotaomicron*–*M. smithii* cocolonization produces a significant increase in host adiposity compared with monoassociated, or *B. thetaiotaomicron*–*D. piger* biassociated, animals. These findings demonstrate a link between this archaeon, prioritized bacterial utilization of polysaccharides commonly encountered in our modern diets, and host energy balance.

adiposity | energy homeostasis | gut microbial ecology | polysaccharide metabolism | *Methanobrevibacter smithii*

The role of Archaea in human health remains unclear (1). One site where their influence may be profound is the gut. Hydrogen-consuming methanogenic archaeons are present in the intestinal tracts of many invertebrate and vertebrate species (2–4). Our adult intestine contains 10 trillion to 100 trillion microbial cells and is dominated by members of just two divisions of Bacteria, the Bacteroidetes and the Firmicutes (5). Archaea, the only known producers of methane, are present at high levels in 50–85% of humans (6–8). Concordance rates for methane production are equivalent for both monozygotic and dizygotic twins, underscoring the importance of family environment (mothers) in acquisition of methanogens (9). The most comprehensive enumeration of the adult human colonic microbiota reported to date (5) found a single predominant archaeal species, *Methanobrevibacter smithii*. This Euryarchaeote can comprise up to 10% of all anaerobes in the colons of healthy adults, while *Methanosphaera stadtmanae* and Crenarchaeotes can be minor members (10, 11).

One manifestation of the mutualism between humans and their distal gut microbiota is that the latter extracts energy that would be lost from otherwise indigestible dietary polysaccharides (fiber). Fermentation of dietary fiber is accomplished by syntrophic interactions between microbes linked in a metabolic food web and is a major energy-producing pathway for members of the Bacteroidetes and the Firmicutes. These primary bacterial fermentors generate short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate, as well as other organic acids (e.g., formate) and gases [e.g., hydrogen (H_2) and carbon dioxide (CO_2)]. Accumulation of H_2 inhibits bacterial NADH dehydrogenases, thereby reducing the yield of ATP. Studies in man-made bioreactors have shown that removal of H_2 by means of archaeal methanogenesis improves fermentation efficiency (12).

Little is known about how our distal gut microbial community prioritizes processing of undigested dietary polysaccharides delivered from the small intestine. We do know that microbial fermentation of these polysaccharides to SCFAs accounts for up

to 10% of our daily caloric intake (13), although the amount will vary depending on the amount of polysaccharides consumed (14), and that, relative to our own genome, our colonic microbial community genome (microbiome) is significantly enriched in components of metabolic pathways involved in glycan degradation (15). We also know that colonization of adult germ-free (GF) mice with an unfractionated gut microbiota harvested from conventionally raised animals produces a prompt and marked increase in adiposity without a corresponding increase in food consumption (16). Together, these observations raise the question of how archaeons, such as *M. smithii*, influence the dining habitats of bacteria living in our guts and how differences in their representation between humans could affect host-energy balance.

To our knowledge, there have been no reported *in vivo* analyses of the impact of a methanogen and a saccharolytic bacterium on one another's transcriptomes or metabolomes. In the present study, we perform such an analysis by examining the effects of *M. smithii* in gnotobiotic mice harboring a simplified two-component model of our colonic microbiota. These experiments indicate that *M. smithii* acts as a “power broker” in the distal gut community, regulating the specificity of polysaccharide fermentation and influencing the amount of calories deposited in fat stores.

Results and Discussion

Cocolonization of Gnotobiotic Mice with *Bacteroides thetaiotaomicron* and *M. smithii* Increases Their Population Density in the Distal Gut. Groups of age-matched adult GF mice belonging to the NMRI inbred strain (17) were colonized with one or more of the following human fecal-derived microbial strains: *B. thetaiotaomicron*, a saccharolytic bacterium whose genome has been sequenced (5, 17, 18) (alone for 14 or 28 d); *M. smithii* (alone for 14 d); or *B. thetaiotaomicron* alone for 14 d followed by *M. smithii* for 14 d.

Sulfate-reducing bacteria (SRB), and to some extent homoacetogens, serve as alternative consumers of H_2 in the human gut (19, 20). These SRBs are almost exclusively *Desulfovibrio* spp., with *Desulfovibrio piger* being the most abundant species in healthy adults (5, 21). *D. piger*, like *M. smithii*, is nonsaccharolytic; unlike *M. smithii*, it cannot use formate (22). Therefore, control experiments were performed where GF mice were colonized with *D. piger* alone or in place of *M. smithii* in biassociation (cocolonization) experiments.

All mice were fed an autoclaved standard rodent chow diet rich in plant polysaccharides (17), including polyfructose-

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Abbreviations: GF, germ-free; SCFA, short-chain fatty acid; qRT-PCR, quantitative RT-PCR.

Data deposition: The *M. smithii* gene sequences reported in this paper have been deposited in the GenBank database (accession no. DQ419923).

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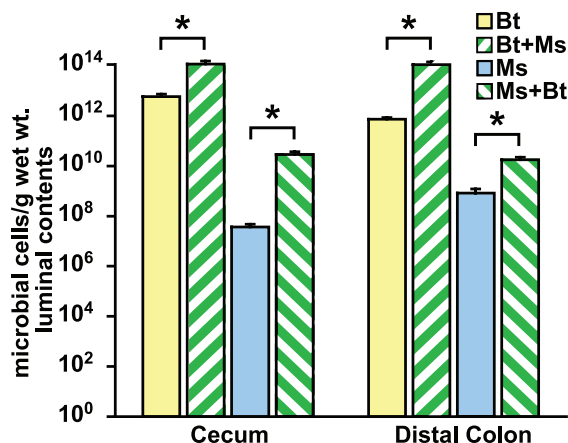


Fig. 1. Cocolonization with *M. smithii* (Ms) and *B. thetaiotaomicron* (Bt) enhances the representation of both species in the distal intestines of gnotobiotic mice. The density of colonization was defined by using quantitative PCR of DNA isolated from the cecal contents of monoassociated and biassociated mice ($n = 5$ per group per experiment; three independent experiments; each sample assayed in triplicate; mean values \pm SEM plotted); *, $P < 0.05$ vs. monoassociated controls. Bt and Bt + Ms represent the number of *B. thetaiotaomicron* cells present in mice colonized with Bt alone or with Bt + Ms, respectively; Ms and Ms + Bt represent the number of *M. smithii* cells present in mice colonized with Ms alone or with Ms + Bt, respectively.

containing glycans (fructans). Biochemical studies of cecal contents recovered from GF mice fed this diet revealed that fructans were 3.8-fold higher than polyglucose-containing glycans (glucans) (85 ± 6 vs. 25 ± 2 $\mu\text{mol/g}$ dry weight of contents; $P < 0.005$).

GF mice were reliably and efficiently colonized after a single gavage of 10^8 *M. smithii* or *B. thetaiotaomicron* (mean values: 10^{12} organisms per g of cecal contents for *B. thetaiotaomicron*; 10^7 for *M. smithii*; Fig. 1). There were no significant differences in cecal *B. thetaiotaomicron* levels after 14- versus 28-d monoassociations (data not shown). Cocolonization with *M. smithii* and *B. thetaiotaomicron* resulted in statistically significant ($P < 0.03$), 100- to 1,000-fold enhancement in the density of cecal colonization by both organisms (Fig. 1). The levels of colonization achieved by *M. smithii* in the ceca and colons (Fig. 1) of these biassociated mice were equivalent to those reported in the feces of healthy adult humans (11). In contrast, biassociation of mice with *B. thetaiotaomicron* and *D. piger* did not significantly alter cecal or colonic levels of either organism (data not shown). These results suggest that a mutually beneficial relationship is forged between *M. smithii* and *B. thetaiotaomicron* in the distal mouse gut that allows them to markedly increase their population size.

***M. smithii* Enhances the Ability of *B. thetaiotaomicron* to Degrade Polyfructose-Containing Glycans.** We used a combination of whole-genome transcriptional profiling, mass spectrometry (MS), and microanalytic biochemical assays to determine the impact of *M. smithii* on *B. thetaiotaomicron* glycan utilization *in vivo*. Total microbial RNA was isolated from cecal contents of monoassociated and biassociated mice ($n = 4$ –5 per group). cDNA targets, generated from each RNA preparation, were hybridized to individual *B. thetaiotaomicron* GeneChips containing probe sets representing 4,719 of *B. thetaiotaomicron*'s 4,779 predicted protein-coding ORFs (17). These probe sets encompass all components of *B. thetaiotaomicron*'s very prominent "glycobiome" (genes involved in carbohydrate acquisition/metabolism/biosynthesis), including 226 predicted glycoside hydrolases, 15 polysaccharide lyases, and 163 paralogs of two outer membrane

proteins (SusC, a malto-oligosaccharide porin, and SusD, which binds starch) (18).

Unsupervised hierarchical clustering of the resulting GeneChip data sets revealed that colonization of the cecal habitat with *M. smithii* dramatically alters *B. thetaiotaomicron*'s transcriptome: 638 genes were defined (see *Materials and Methods*) as significantly up-regulated, and 462 genes were defined as significantly down-regulated compared with their levels of expression during a 14-d *B. thetaiotaomicron* monoassociation [$P < 0.05$; see Table 1, which is published as supporting information on the PNAS web site, for a gene list sorted by clusters of orthologous groups (COGs)]. Consistent with the enhanced representation of *B. thetaiotaomicron* in the distal gut, our COG-based functional classification indicated that cocolonization with *M. smithii* up-regulates *B. thetaiotaomicron* genes involved in DNA replication and protein production (see Fig. 5, which is published as supporting information on the PNAS web site). The presence of *M. smithii* also causes *B. thetaiotaomicron* to down-regulate expression of many genes involved in carbohydrate metabolism (Fig. 5), including 57 glycoside hydrolases [e.g., arabinosidases, xylosidases, glucosidases, galactosidases, mannosidases, rhamnosidases, and pectate lyases; grouped by carbohydrate-active enzyme families (CAZy; <http://afmb.cnrs-mrs.fr/CAZY/acc.html>) in Table 2, which is published as supporting information on the PNAS web site]. There is an accompanying induction of three fructofuranosidases (Fig. 24). Two of these polyfructose-degrading glycoside hydrolases are encoded by ORFs situated in a gene cluster (BT1757–BT1765) that includes a putative sugar transporter, SusC/SusD paralogs, and the organism's only fructokinase (Fig. 2B). Augmented expression of this cluster was validated by quantitative RT-PCR (qRT-PCR): the results revealed 32 ± 5.8 - and 47 ± 5.9 -fold increases for the fructofuranosidases (BT1759 and BT1765, respectively) and a 6.4 ± 2.8 -fold increase for the fructokinase (BT1757) (see Fig. 6A and Tables 3 and 4, which are published as supporting information on the PNAS web site). Changes in the fructofuranosidases represent the most robust *B. thetaiotaomicron* transcriptional responses to *M. smithii*.

In contrast, cocolonization with *D. piger* did not produce a significant change in expression of these fructofuranosidases, or the fructokinase, as judged by GeneChip and qRT-PCR assays (Fig. 6 and Table 3). Overall, *D. piger* had very modest effects on the *B. thetaiotaomicron* transcriptome: of the 41 differentially expressed genes, only four were glycoside hydrolases (two α -mannosidases, a β -hexosaminidase, and a glucuronyl hydrolase; all down-regulated; see Fig. 6B).

Fructose is easily shunted into the glycolytic pathway by means of fructokinase, making fructans desirable energy sources. This notion is supported by GeneChip analyses of *B. thetaiotaomicron* grown in a batch culture fermentor containing glucose and a complex mixture of polysaccharides (TYG medium). Expression of the polyfructose degradation cluster peaks in early log phase with 7.5- to 53.2-fold higher levels for BT1757–BT1765 transcripts compared with late log/stationary phase where *B. thetaiotaomicron* utilizes less coveted glycans such as mannans (ref. 17; data sets are available from the authors upon request).

Consistent with the *in vitro* and *in vivo* transcriptional profiling results, biochemical assays demonstrated a statistically significant $52 \pm 4\%$ decrease in cecal fructan levels after *B. thetaiotaomicron*/*M. smithii* cocolonization, compared with *B. thetaiotaomicron* monoassociated mice ($P < 0.05$; Fig. 2C). Glucans increased modestly ($15 \pm 3\%$; $P < 0.05$; Fig. 2C), indicating continued albeit slightly reduced digestion of glucose-containing polysaccharides. In contrast, cocolonization with *D. piger* did not produce any significant changes in fructan or glucan levels compared with the *B. thetaiotaomicron* monoassociated state (data not shown).

GC-MS analysis of neutral and amino sugars released by acid

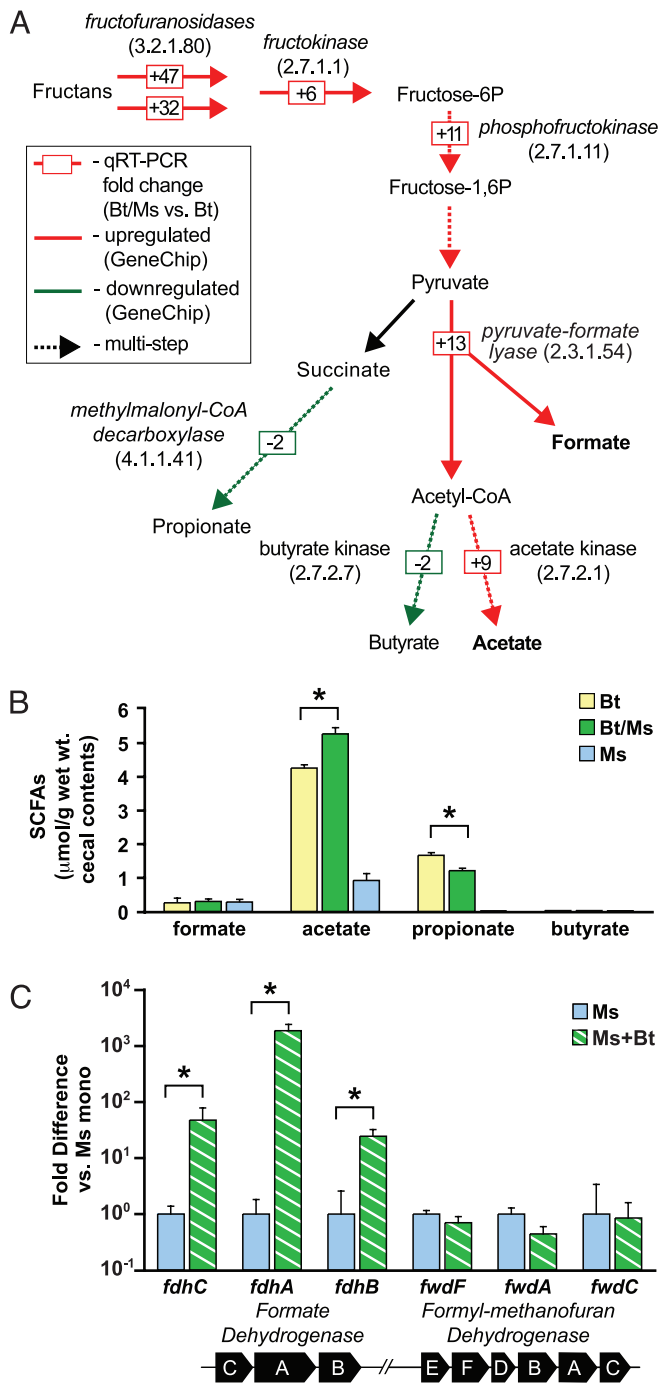


Fig. 3. Biassociation with *B. thetaiotaomicon* and *M. smithii* increases *B. thetaiotaomicon* production of acetate and formate. (A) qRT-PCR analysis (boxed numbers) of the effects of *M. smithii* on expression of selected *B. thetaiotaomicon* genes encoding enzymes involved in fermentation of polyfructose-containing glycans: fructofuranosidases, BT1765/BT1759; fructokinase, BT1757; phosphofructokinase, BT0307; pyruvate:formate lyase, BT4738; acetate kinase, BT3963, methylmalonyl-CoA decarboxylase, BT1688; butyrate kinase, BT2552. Enzyme classification (EC) numbers are provided in parentheses. Dotted lines indicate multistep pathways. (Expression of fructofuranosidases, acetate kinase, pyruvate:formate lyase, and butyrate kinase is constant if the colonization period is extended from 14 to 28 d; see Table 3.) (B) GC-MS analyses of cecal SCFAs ($n = 5$ per group; each sample assayed in duplicate; mean values \pm SEM plotted; *, $P < 0.05$). (C) qRT-PCR study of the *in vivo* expression of *M. smithii* genes in a cluster (Lower) containing a formate transporter and dehydrogenase (*fdhCAB*) plus tungsten-containing formylmethanofuran dehydrogenase subunits (*fwdeFDBAC*) ($n = 5$ per group; each sample assayed in triplicate; mean values \pm SEM plotted; *, $P < 0.05$).

that *M. smithii* transcripts encoding FdhC, FdhA, and FdhB were expressed at 48 ± 3 -fold, $1,882 \pm 559$ -fold, and 25 ± 8 -fold higher levels, respectively, when *B. thetaiotaomicron* was present. Formyl-methanofuran dehydrogenase was constitutively expressed and not affected by biassociation (Fig. 3C).

These findings reveal some of the underpinnings of *M. smithii*–*B. thetaiotaomicron* mutualism. *B. thetaiotaomicron* obtains energy from facilitated fermentation of coveted glycans (fructans) and increased production of acetate (yields more ATP than other end products of fermentation; ref. 12). This process allows a larger population of *B. thetaiotaomicron* to be supported (Fig. 1). *M. smithii*, in turn, benefits by obtaining formate from *B. thetaiotaomicron* for methanogenesis, and its population expands accordingly (Fig. 1).

Ability of the Host to Harvest and Store Calories from the Diet Is Enhanced by *M. smithii*. The host also benefits. As noted above,

colonic absorption of SCFAs generated during fermentation represents $\approx 10\%$ of the daily caloric intake from a Western diet (13). *B. thetaiotaomicron*/*M. smithii* biassociated mice exhibit increased production and absorption of SCFAs. As in the cecum, addition of *M. smithii* produced significantly greater serum acetate levels compared with *B. thetaiotaomicron* monoassociated controls; in contrast, no significant increases occurred with addition of *D. piger* (Fig. 4A). Distal gut-derived SCFAs are transported, via the portal vein, to the liver where they stimulate *de novo* lipogenesis. Fatty acid synthase (*Fas*) is a key enzyme in this pathway; qRT-PCR studies showed that hepatic *Fas* gene expression was increased $142 \pm 13\%$ in *B. thetaiotaomicron*/*M. smithii* vs. $61 \pm 9\%$ in *B. thetaiotaomicron* monoassociated mice ($P < 0.03$). Biochemical assays confirmed that addition of *M. smithii*, but not *D. piger*, to *B. thetaiotaomicron*-colonized animals produced significant increases in total liver triglyceride levels (Fig. 4B).

The increase in hepatic *de novo* lipogenesis was accompanied by increased storage of energy in fat cells. Epididymal fat pad weights were significantly greater in *B. thetaiotaomicron*/*M. smithii* biassociated mice compared with *B. thetaiotaomicron* monoassociated controls [$80 \pm 6\%$ increase over GF vs. $54 \pm 7\%$; $P < 0.05$; Fig. 4C]. In contrast, there was no significant difference in fat pad weights between the *B. thetaiotaomicron*/*D. piger* and *B. thetaiotaomicron* groups (Fig. 4C). Dual-energy x-ray absorptiometry independently confirmed these findings: compared with GF mice, total body fat stores were increased by $47 \pm 4\%$ in *B. thetaiotaomicron*/*M. smithii* biassociated vs. $34 \pm 3\%$ in *B. thetaiotaomicron* monoassociated animals ($n = 5$ per group; $P < 0.05$). The increase in adiposity was not accompanied by any statistically significant differences in chow consumption (data not shown). Total body weight did not change significantly (data not shown), a finding explained by the reduction in cecal weight that is well known to occur after colonization of gnotobiotic animals (26).

Prospectus

The present work indicates that the representation of methanogenic archaea in an individual's gut microbiota affects energy harvest from dietary glycans as well as host energy storage. The caloric content of food items is typically portrayed as a fixed value on package labels irrespective of differences that exist in the microbial ecology of our intestines (5, 27). Moreover, although metabolic studies indicate that high-fat diets are more likely to result in increased adiposity than high-carbohydrate diets, a common view is that different types of carbohydrates do not differ significantly with respect to their impact on energy balance (28). Our findings suggest that these views should be revisited. We are approaching an era of personalized nutrition where the energy content of our diet should and can be matched to the fermentative capacity of our intestinal microbiota. For

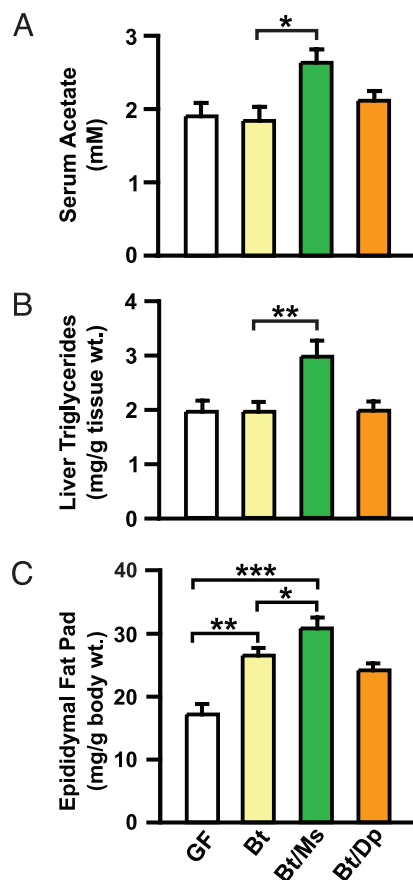


Fig. 4. Cocolonization of mice with *M. smithii* and *B. theta*otaomicon enhances host energy storage. (A) GC-MS analyses of acetate in sera obtained by retro-orbital phlebotomy from fasted (4 h) 12-week-old male GF, *B. theta*otaomicon monoassociated, and bioassociated [*B. theta*otaomicon/*M. smithii* or *B. theta*otaomicon/*D. piger* (Dp)] gnotobiotic mice ($n = 5$ per group per experiment; two independent experiments; mean \pm SEM are plotted). (B) Liver triglyceride levels ($n = 5$ per group; each assayed in duplicate; mean \pm SEM plotted). (C) Epididymal fat pad weights ($n = 5$ per group per experiment; two independent experiments; mean \pm SEM plotted). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

example, fructans are commonly consumed as wheat products (29) and, increasingly, as sweeteners (30). Our simplified gnotobiotic model of the human gut ecosystem indicates that the presence of *M. smithii* may enhance the ability to extract calories from this class of polysaccharides.

Further studies are needed to understand how to manipulate the representation of *M. smithii* and/or other archaeons in our gut microbiota: the results could lead to a novel means for preventing obesity in the overfed or increasing caloric harvest in the underfed. Gnotobiotic mice harboring intentionally created communities of bacteria and archaea may be helpful in identifying interventions that could be used to test this concept in humans.

Materials and Methods

Colonization of GF Mice. Mice belonging to the NMRI/KI inbred strain (31) were housed in gnotobiotic isolators (32) where they were maintained on a strict 12-h light cycle (lights on at 0600 h) and fed a standard autoclaved polysaccharide-rich chow diet (B & K Universal, East Yorkshire, U.K.) ad libitum. Each mouse was inoculated with a single gavage with 10^8 microbes per strain (harvested from overnight stationary phase cultures in the case of *B. theta* and *B. omicron*, 4-d cultures for *D. piger*, and from serum bottles after a 5-d incubation for *M. smithii*). Within a given

experiment, the same preparation of cultured microbes was used for biassociation and monoassociation. Details of how colonization levels were determined are described in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

RNA Isolation and GeneChip Analysis. A total of 100–300 mg of frozen cecal contents from each gnotobiotic mouse was added to 2-ml tubes containing 250 μ l of 212- to 300- μ m-diameter acid-washed glass beads (Sigma), 500 μ l of Buffer A (200 mM NaCl/20 mM EDTA), 210 μ l of 20% SDS, and 500 μ l of a mixture of phenol:chloroform:isoamyl alcohol (25:24:1; pH 4.5; Ambion, Austin, TX). Samples were lysed by using a bead beater (BioSpec Products, Bartlesville, OK; “high” setting for 5 min at room temperature). Cellular debris was pelleted by centrifugation (10,000 \times g at 4°C for 3 min). The extraction was repeated by adding another 500 μ l of phenol:chloroform:isoamyl alcohol to the aqueous supernatant. RNA was precipitated and resuspended in 100 μ l of nuclease-free water (Ambion); 350 μ l of Buffer RLT (Qiagen, Valencia, CA) was added, and RNA was further purified by using the Qiagen RNeasy mini kit. cDNA targets for GeneChip hybridization were prepared, according to the manufacturer’s instructions (Affymetrix, Santa Clara, CA), from cecal microbial RNA samples isolated from each mouse in each treatment group and then hybridized to individual custom Affymetrix *B. theta*taoamicon GeneChips (17). Details of qRT-PCR-based analyses of selected transcripts are described in *Supporting Materials and Methods*.

All GeneChip data sets were analyzed by using DNA-CHIP ANALYZER V1.3 (DCHIP; www.biostat.harvard.edu/complab/dchip) and methods described by Sonnenburg *et al.* (17). Normalized and modeled (PM-MM) data sets were generated and used to identify differentially expressed genes between the experimental (E) and baseline (B) groups based on the following criteria: $E - B > 50$; $E = B$; $P < 0.05$; $\geq 33\%$ “Present” call in B; $\geq 66\%$ “Present” call in E; false discovery rate $< 3\%$. *In silico* metabolic reconstructions were performed by using METAVIEW (33) as described (17). Reconstructions based on the data sets referenced in this study are available from the authors upon request.

Analysis of Cecal Glycans. GC-MS was used for determining neutral and amino sugars in cecal glycans (16). Fructan levels were assayed by using a different microanalytic approach (34). Cecal samples were collected, freeze dried at -35°C for 4 d, weighed, and stored under vacuum at -80°C until use (stable for at least 1 month). Samples (10–15 mg) then were homogenized at 1°C in 0.25 ml of 1% oxalic acid (prepared in H_2O) and divided into two equal-sized aliquots, one of which was heated to 100°C for 30 min (acid hydrolysis sample), whereas the other was maintained at 1°C (control sample). A $10\text{-}\mu\text{l}$ aliquot of each sample was added to a 1-ml solution containing 50 mM Tris-HCl (pH 8.1), 1 mM MgCl_2 , 0.02% BSA, 0.5 mM ATP, 0.1 mM NADP^+ , 2 $\mu\text{g/ml}$ *Leuconostoc mesenteroides* glucose-6 phosphate dehydrogenase (253 units/mg protein; Calbiochem), 10 $\mu\text{g/ml}$ yeast hexokinase (50 units/mg protein; Sigma), and 10 $\mu\text{g/ml}$ yeast phosphoglucose isomerase (500 units/mg protein; Sigma). The mixture subsequently was incubated for 30 min at 24°C . The resulting NADPH product was detected by using a fluorimeter. Glucan levels were measured in a similar manner as that for fructans, except that phosphoglucose isomerase was omitted from the reactions. Fructose and glucose standards (5–10 nmol) were carried through all steps.

Assays of Organic Acid Levels. SCFAs in mouse serum and cecal samples were assayed by using a modification of the method of Moreau *et al.* (35). For analysis of sera, mice were fasted for 4 h; blood was collected by retro-orbital phlebotomy into serum separation tubes (Becton Dickinson) and spun; and the super-

natant (serum) was stored at -80°C before assay. Fifty microliters of serum, or 100–200 mg of frozen cecal contents, was transferred to a 4-ml glass vial fitted with a septum cap polytetrafluoroethylene (PTFE) liner (National Scientific, Rockwood, TN) and containing 10 μl of stock solution of internal standards (Isotec; each of the following components at 20 mM: $[^2\text{H}_2]$ - and $[1-^{13}\text{C}]$ acetate, $[^2\text{H}_5]$ propionate, and $[^{13}\text{C}_4]$ butyrate). After acidification with 10 μl of 37% HCl, SCFAs were extracted (2 ml diethyl ether/extraction; 2 cycles). An aliquot of each sample then was derivatized with *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA; Sigma), and SCFAs were quantified by using a gas chromatograph (Model 6890; Hewlett-Packard) coupled to a mass spectrometer detector (Model 5973; Agilent Technologies, Palo Alto, CA) as described in *Supporting Materials and Methods*.

Analysis of Host Energy Storage. Total body fat content was measured in 12-week-old male NMRI mice by using dual-

energy x-ray absorptiometry (Lunar PIXImus Mouse; GE Medical Systems, Waukesha, WI) as described (16, 36). Epididymal fat pads and livers were removed and weighed. A portion of the liver was assayed for triglyceride content according to a standard biochemical method described by Marshall *et al.* (37).

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